B1

proteins that control gene transcription through interactions with specific gene sequences. --

Please replace the paragraph beginning at page 8, line 29, and ending at page 8, line 32, with the following rewritten paragraph:

B2 -- A further aspect of the invention provides a purified androgen receptor protein and purified polypeptides and proteins have substantially the same biological activity as androgen receptor protein, and methods for producing such proteins and polypeptides.--

Please replace the paragraphs beginning at page 9, line 2, and ending at page 10, line 23, with the following rewritten paragraphs:

B3 - - Figure 1 shows a comparison of DNA-binding domains of the human androgen receptor (hAR) with members of the nuclear receptor family. (A) is a comparison of oligo A nucleotide sequence (**SEQ ID NO:1**) with sequences of hAR (**SEQ ID NO:2**) and other nuclear receptors: hPR, human progesterone receptor (**SEQ ID NO:3**); hMR, human mineralocorticoid receptor (**SEQ ID NO:4**); hGR, human glucocorticoid receptor (**SEQ ID NO:5**); hER, human estrogen receptor (**SEQ ID NO:6**); hT3R, human thyroid hormone receptor (**SEQ ID NO:7**); hRAR, human retinoic acid receptor (**SEQ ID NO:8**). Chromosomal locations are shown in parentheses at the left. Nucleotide identity between oligo A and hAR is indicated with an asterisk. The percent homology with oligo A is in parentheses at the right of each sequence. (B) shows the structure of fibroblast clone ARHFL1 human fibroblast clone [1]. Nucleotide residues are numbered from the 5'-terminus. Restriction endonuclease sites were determined by mapping or were deduced from DNA sequence. The TGA translation termination codon, determined by comparison with hPR, hMR and hGR, follows a long open reading frame containing sequences homologous to those of other steroid receptors. Arrows indicate exon boundaries in genomic clone X05AR. The hatched area is the putative DNA-binding domain. (C) shows a comparison of amino acid

B3 sequences of the AR DNA-binding domain (**SEQ ID NO:9**) with sequences of the nuclear receptor family. AR amino acid sequence was deduced from nucleotide sequence of clone ARHFL1 and is numbered beginning with the first conserved cysteine residue (+). Amino acid numbers in parentheses at the left indicate the residue number of the first conserved cysteine from the references indicated below. Percent homology with hAR is indicated in parentheses on the right. The region of the DNA-binding domain from which the oligo A sequence was derived is underlined in hAR. Coding DNA of residues 1 to 31 is contained within genomic clone X05AR. Abbreviations are hPR, human progesterone receptor (**SEQ ID NO:10**); hMR, human mineralocorticoid receptor (**SEQ ID NO:11**); hGR, human glucocorticoid receptor (**SEQ ID NO:12**); hER, human estrogen receptor (**SEQ ID NO:13**); cVDR, chicken vitamin D receptor (**SEQ ID NO:14**); hT3R, human thyroid hormone receptor (**SEQ ID NO:15**); vERBA, erb A protein from avian erythroblastosis virus (**SEQ ID NO:16**); and hRAR, human retinoic acid receptor (**SEQ ID NO:17**). Abbreviations for amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Figure 2 illustrates the steroid binding properties of expressed AR cDNA. (A) shows the structure of pCMVAR in the expression vector pCMV containing the human cytomegalovirus (CMV) promoter of the immediate early gene, poly(A) addition-transcription terminator region of the human growth hormone gene (hGH poly A), SV40 origin of replication (SV40 Ori), and a polylinker region for insertion of cDNAs. The plasmid pTEBR contains the ampicillin resistance gene (Amp). (B) shows saturation analysis of [^{3}H]dihydrotestosterone binding in extracts of pCMVAR transfection of COS M6 cells. Portions of cytosol (0.1 ml, 0.3 mg/ml protein) were incubated overnight at 4°C with increasing concentrations of ^{3}H-labeled hormone and analyzed by charcoal adsorption. Nonspecific binding increased from 18% to 37% of total bound radioactivity. (C) shows a Scatchard plot analysis of [^{3}H]dihydrotestosterone binding. Error estimation was based on linear

B3 regression analysis (r=0.966). (D) illustrates the competition of unlabeled steroids for binding of 5 nM [^{3}H]dihydrotestosterone in transfected COS M6 cell extracts. Unlabeled steroids were added at 10- and 100-fold excess of labeled hormone. Specific binding was determined as previously described. --

Concluded

Please replace the paragraphs beginning at page 10, line 31, and ending at page 11, line 5 with the following rewritten paragraphs:

B4 B4 -- Figure 4A-I shows the double-stranded DNA sequence (**SEQ ID NO:18**) encoding the human androgen receptor protein.

Figure 5A-E shows the complete single-stranded DNA sequence (5082 bases) of the human androgen receptor (**SEQ ID NO:18**) and the deduced amino acid sequence (**SEQ ID NO:19**). No intron sequence is included.

Figure 6A-6D shows the complete single-stranded DNA sequence (4260 bases) of the rat androgen receptor (**SEQ ID NO:20**) and the deduced amino acid sequence (**SEQ ID NO:21**). --

The paragraph beginning at page 11, line 6, and ending at page 11, line 11, has been deleted.

Please replace the paragraphs beginning at page 11, line 12, and ending at page 11, line 16, with the following rewritten paragraphs:

B5 -- Figure 7 is a photograph showing restriction fragment length polymorphisms in the human androgen receptor gene.

Figure 8 is a photograph showing a Southern blot analysis in the human androgen receptor gene in complete androgen insensitivity syndrome patients.--

Please replace the paragraphs beginning at page 12, line 9, and ending at page 12, line 20, with the following rewritten paragraphs:

B6 -- The grouping of codons during translation of mRNA into amino acid sequences. During translation the proper reading frame must be maintained.

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For example, the sequence GCTGGTTGTAAG (**SEQ ID NO:22**) may be translated in three reading frames or phases, each of which affords a different amino acid sequence:

GCT GGT TGT AAG – Ala-Gly-Cys-Lys (**SEQ ID NO:23**)

G CTG GTT GTA AG – Leu-Val-Val

GC TGG TTG TAA A – Trp-Leu-(Stop)

Polypeptide

A linear series of amino acids connected one to the other by peptide bonds between the α-amino and carboxy groups of adjacent amino acids. --

Please replace the paragraphs beginning at page 14, line 28, and ending at page 15, line 19, with the following rewritten paragraphs:

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-- A human X chromosomal library was screened with the synthetic oligonucleotide probe A (Oligo A sequence = $^{5'}$ CTT TTG AAG AAG ACC TTA CAG CCC TCA CAG GT $^{3'}$; **SEQ ID NO:24**) of Figure 1 (A) designed as a consensus sequence from the conserved sequence of the DNA-binding domain of other steroid receptors. Screening the library with the oligo A probe resulted in several recombinants whose inserts were cloned into bacteriophage M13 DNA and sequenced. One recombinant clone (Charon 35 X05AR) (human genomic clone [1]) contained a sequence similar to, yet distinct from, the DNA-binding domains of other steroid receptors. It had 84% sequence identity with oligo A, while other receptor DNAs were 78% to 91% homologous with consensus oligonucleotide.

From the nucleotide sequence just 5' of the DNA-binding domain, oligonucleotide probe B (Oligonucleotide B sequence = $^{5'}$ GGA CCA TGT TTT GCC CAT TGA CTA TTA CTT TCC ACC CC $^{3'}$; **SEQ ID NO:25**) was synthesized and used to screen bacteriophage lambda gt11 cDNA libraries from human epididymis and cultured human foreskin fibroblasts. Recombinant phage (unamplified) screened with this oligonucleotide by in situ hybridization revealed one positive clone in each library. The epididymal clone (gt11 ARHEL1) (human epididymis clone [1]) contained the complete

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DNA-binding domain and approximately 1.5 kb of upstream sequence, whereas the fibroblast clone (gt11 ARHFL1) (human fibroblast clone [1]) shown in Figure 1(B) contained the DNA-binding domain and 1.5 kb of downstream sequence. The DNA-binding domains of the cDNA isolates were identical to that of the genomic exon sequence. --

Please replace the paragraphs beginning at page 16, line 32, and ending at page 17, line 29, with the following rewritten paragraphs:

B8

-- The complete double-stranded sequence (**SEQ ID NO:18**) encoding the human androgen receptor protein was determined and is set forth in Figure 4. The single-stranded DNA sequence (**SEQ ID NO:18**) encoding human androgen receptor protein along with the amino acid sequence (**SEQ ID NO:19**) which it codes for are set forth in Figure 5. The single stranded DNA sequence (**SEQ ID NO:20**) and the amino acid sequence (**SEQ ID NO:21**) for the rat androgen receptor protein is set forth in Figure 6.

Recombinant DNA human fibroblast clone [1] isolated from human foreskin fibroblast cDNA gt11 expression library, human epididymis clones [1] and [5] isolated from human epididymis cDNA gt11 expression library were deposited in the American Type Culture Collection with accession numbers ATCC # 40439, ATCC # 40442 and ATCC # 40440, respectively. Human genomic clones [1], [2], [3], [4] and [5] which were isolated from human X chromosome lambda Charon 35 library available as ATCC # 57750 have been deposited with the American Type Culture Collection with accession numbers ATCC # 40441, ATCC # 40443, ATCC # 40444, ATCC # 40445 and ATCC # 40446, respectively.

A wide variety of host-cloning vehicle combinations may be usefully employed in cloning the double-stranded DNA disclosed herein. For example, useful cloning vehicles may include chromosomal, non-chromosomal and synthetic DNA sequences such as various known bacterial plasmids and wider host range plasmids such as pCMV and vectors derived from combinations of plasmids and phase DNA such as plasmids which have been

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modified to employ phage DNA expression control sequences. Useful hosts may include bacterial hosts, yeasts and other fungi, animal or plant hosts, such as Chinese Hamster Ovary Cells (CHO), or monkey kidney cells (COS M6), and other hosts. The particular selection of host-cloning vehicle combinations may be made by those of skill in the art after due consideration of factors such as the source of the DNA- i.e. genomic or cDNA. --

Please replace the paragraph beginning at page 18, line 3, and ending at page 18, line 8, with the following rewritten paragraph:

B9 -- Techniques for transforming hosts and expressing foreign cloned DNA in them are well known in the art (See, for example, Maniatis et al., infra). Cloning vehicles used for expressing foreign genes in bacterial hosts will generally contain a selectable marker, such as a gene for antibiotic resistance, and a promoter which functions in the host cell. --

Please replace the paragraph beginning at page 20, line 24, and ending at page 20, line 33, with the following rewritten paragraph:

B10 -- Proteins and peptides of this invention can be utilized for the production of polyclonal or monoclonal antibodies. Methods for production of such antibodies are known to those of ordinary skill in the art and may be performed without undue experimentation. One method for the production of monoclonal antibodies is described in Kohler, G., et al., "Continuous Culture of Fused Cells Secreting Antibody of Predefined Specificity," Nature, vol. 256 (1975), p. 495, which is incorporated herein by reference. Polyclonal antibodies, by way of example, can be produced by the method described below. --

Please replace the paragraph beginning at page 21, line 5, and ending at page 21, line 17, with the following rewritten paragraph:

B11 -- For example, one androgen receptor sequence, NH_2-Asp-His-Val-Leu-Pro-Ile-Asp-Tyr-Tyr-Phe-Pro-Pro-Gln-Lys-Thr (**SEQ ID NO:26**) in the 5'

B11

region upstream from the DNA-binding domain, was used to raise antisera in rabbits. The antisera react selectively at a dilution of 1 to 500 with the androgen receptor both in its untransformed 8-10S form and in its 4-5S transformed form. Receptor sedimentation on sucrose gradients increases from 4 to 8-10S in the presence of antiserum at high ionic strength and from 8-10S to 11-12S at low ionic strength sucrose gradients. In the ELISA reaction against the peptide used as immunogen, reactivity was detectable at 1 to 25,000 dilution. This antiserum at a dilution of 1 to 3000 was found effective in staining nuclear androgen receptor in rat prostrate and other male accessory sex glands (data not shown). --

Please replace the paragraph beginning at page 22, line 9, and ending at page 22, line 14, with the following rewritten paragraph:

B12

-- In addition, small mutations can be detected utilizing methods known to one of ordinary skill in the art, from cultured skin fibroblasts of the affected individual. A cDNA library can be prepared using standard techniques. The androgen receptor clones can be isolated using a [^{32}P]DNA AR probe. The AR cDNA clones can then be sequenced and compared to normal AR cDNA sequences. --

Please replace the paragraphs beginning at page 23, line 10, to page 23, line 33, with the following rewritten paragraphs:

B13

-- For example, a human restriction fragment length was determined by cDNA probe (B) and Hind III restriction endonuclease using the Southern blot technique (See Figure 7). The two RFLP alleles detected are a fragment at 6.5 kb (allele 1) and a fragment at 3.5 kb (allele 2). Major constant fragment bands are seen at approximately 2 and 5 kb with minor constant bands at 0.9 and 7.5 kb. Allele 1 is present in approximately 30% of the X chromosomes of the Causasian population. Allele 2 is present in approximately 20% of the X chromosomes of the Causasian population. In Figure 8 Lanes A, B and D, DNA from women who are homozygous for allele 1 is shown. In Figure 8

BB

Lane C, DNA from a woman who is heterozygous for both alleles 1 and 2 is shown. Figure 8 Lane E contains DNA from a man that only possesses allele 2. This RFLP, and others determined by the clones we have isolated, will enable one to monitor the androgen receptor gene in various disease conditions described herein.

An example of using the androgen receptor clones to detect mutations is shown in Figure 8 where five different complete androgen insensitive patients' DNA are digested with EcoRI, electrophoresed, Southern blotted, and probed with cDNA probe B. The patient in lane B lacks a 3 kb band indicating that part of the androgen receptor gene is deleted. Further analysis of this and other patients DNA is possible with other AR probes and by sequencing by standard methods and comparing the abnormal sequence to the normal sequence described herein. --

Concluded